Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 2, 2025, 88
https://doi.org/10.33263/LIANBS142.088

Biocompatible Copper Oxide Nanoparticles Functionalized by Aqueous Pomegranate Peel Extract: Characterization and Their Antibacterial Activities

Wanisa Abdussalam-Mohammed [1,*], Moussa Khlifa [2], Alshafei M. A. Aljarani [3], Abudelrhman Farj [2], Mohamed A. A. Alwahsh [2]

[1] Chemical Engineering Department, Faculty of Engineering, University of Tripoli. Tripoli, Libya; w.ahweelat @uot.edu.ly
[2] Chemistry Department, Faculty of Science, Sebha University, Sebha, Libya; mou.khlifa72@gmail.com (M.K); Abu.mohamed@sebhau.edu.ly (A.F.); moh.alwahsh@sebhau.edu.ly (M.A.A.);
[3] Botany Department, Faculty of Science, Sebha University, Sebha, Libya; als.aljarani@sebhau.edu.ly (A.A.A);
* Correspondence: w.ahweelat@uot.edu.ly;
Scopus Author ID 571892660258

Received: 17.01.2024; Accepted: 30.06.2024; Published: 15.02.2025

Abstract: Copper Oxide Nanoparticles (CuONPs) were synthesized using pomegranate peel aqueous extract as a capping agent and reducing agent in the presence of ascorbic acid. The produced CuONPs were characterized using UV-visible absorption spectroscopy (UV-Vis), Fourier transform infrared (FT-IR), Transmission electron microscopy (TEM), Dynamic light scattering (DLS), and zeta potential. The CuONPs displayed good stability over a period of 6 months. TEM images of CuONPs showed monodispersity and a spherical shape with a small size (19±1.2 nm). Its size was confirmed as well by using DLS and was 33.2±5 nm. Zeta potential revealed that CuONPs have a negative surface charge (-16 mV). The UV-vis confirmed the formation of CuONPs, and the absorption band was observed at 281nm at pH 3.5. The functional groups of active components present in the extract were confirmed by FTIR analysis. The CuONPs were tested for antibacterial effects with Gram-negative bacteria (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria at different concentrations. In both types, high antibacterial activity was obviously observed. These CuONPs can be used in biomedical applications in the future to overcome conventional drug resistance.

Keywords: copper oxide nanoparticles; stability; ascorbic acid; pomegranate peel aqueous extract.
Abbreviation: CuONPs: copper oxide nanoparticles; UV-Vis: UV-Visible absorption spectroscopy; TEM: Transmission electron microscopy; DLS: Dynamic light scattering; *E. coli: Escherichia coli*; S. aureus: *Streptococcus aureus*.

1. Introduction

Nanotechnology has gotten much attention in physics, chemistry, biology, nanomedicine, and electronics. Nanoparticles exhibit unique chemical and physical properties and represent a progressively important material in developing novel nanodevices that can be used in various biological, pharmaceutical, and biomedical applications [1].

Metal and metal oxide nanoparticles have been commonly used in every branch of medicine due to their ability to deliver drugs in the optimum dosage range, usually resulting in increased therapeutic efficiency, declining side effects, and enhanced patient compliance. Antimicrobial agents are vital in textiles, medicine, water disinfection, and food packaging. Consequently, the antimicrobial properties of inorganic NPs add efficacy to this important aspect compared to organic compounds, which are quite toxic to biological systems, as mentioned in the literature [1, 2]. CuONPs have demonstrated promise as possible antioxidants, anticancer agents, antibacterial agents, and drug carriers. They are also used in tissue engineering and disease diagnostics [3].

The importance of CuONPs in medical applications is due to their powerful antibacterial activity and promise as disinfectants against nosocomial infections. In addition, CuONPs are widely used in nanomedicine because of their potent bactericidal properties against various Gramme (+ve(and Gramme)-ve(bacterial strains [4].

CuONPs provide good compatibility with biomolecules. They can be produced with unusual crystal morphologies with remarkably large surface areas, which makes them beneficial in detecting and treating cancerous illness, as illustrated in the later study [5].

Various physiochemical methods have been broadly used to prepare CuONPs [6]. However, these methods contain toxic chemicals that are harmful to the environment and humans due to the release of different hazardous chemicals. They also thought it would be expensive and time-consuming [5, 7]. Consequently, growing environment-friendly protocols and inexpensive methods have become important in nanomaterial synthesis. Plant extracts continue to be the only realistic source of metal and metal oxide nanoparticles due to their rapid reaction rate with low energy, the presence of numerous biomolecules, cost-effectiveness, good stability, safety, absence of hazardous chemicals, and simple operation procedures [8]. For example, plant extracts contain biomolecules such as flavonoids, proteins, phenols, and terpenoids that effectively reduce and stabilize agents for the synthesis of CuONPs [8, 9].

This study synthesized a green approach to CuONPs using pomegranate peel extract as a stabilized ligand and reducing agent in the presence of ascorbic acid.

Pomegranate peel is regarded as a member of the Punicaceae family. Where Pomegranate peel contains tannins, flavonoids, polyphenols, and some anthocyanins such as cyanidins and delphinidins. Extracts of the pomegranate peels have been considered vital in various pharmacological activities [10, 11]. In addition, pomegranate peel is an agro-industrial waste rich in phenolic compounds that can be used in the food and pharmaceutical industries as natural antioxidants [10, 12].

The pomegranate's peel, seeds, and leaves, among other parts, have been associated in several studies with biological activities and therapeutic effects; among the stated properties are the antibacterial, antioxidant, anthelmintic, and anti-inflammatory actions. The most varied uses are those made for pomegranate peel extracts [13].

Furthermore, pomegranate peel is an agro-waste constituting more than half of the weight of the fruit, and it is an abundant source of vitamins (A, B6, B9, and E), as well as potassium and oxalic acid [13].

One of the important goals behind continuous research is the development of novel alternatives to conventional medications resistant to certain types of bacteria, which subsequently increase the potential of new medicines to fight against microbial infections. For this reason, it is necessary to synthesize CuONPs using inexpensive and secure methods to be employed as an alternative in various applications.

2. Materials and Methods

Copper (II) chloride ($CuCl_2$) and ascorbic acid were purchased from (Sigma-Aldrich in the United States of America). All the solutions in the present study were prepared using deionized double-distilled water (DI).

2.1. Characterization.

2.1.1. Ultraviolet-visible spectroscopy (UV-vis).

UV-vis spectra were recorded using an Evolution 300 spectrophotometer with a double beam principal system, and data was recorded using the Vision software version on Windows XP/2000. (Biochrom, Cambridge, UK).

2.1.2. Fourier transform infrared spectroscopy (FT-IR) analysis.

Fourier transform infrared spectroscopy (Thermo Nicolet 380) was used to identify the functional groups in CuONPs samples and pomegranate peel extract. The FTIR spectra of the CuONPs and extract were recorded in the 400 to 4000 cm−1 range with a resolution of 4 cm^{-1}. Origin software (Version 7.5) was used to and analyze the spectra.

2.1.3. Transmission electron microscopy (TEM).

Samples were prepared for TEM micrographs by dropping a CuONP solution onto a carbon-coated copper grid (S160-3 Plano GmbH). The samples were then analyzed using a JEOL 120/JEOL 200 TEM (Carl Zeiss NTS) with a 120 kV/200 kV setting. The photos were enhanced in contrast by applying zero-loss energy filtering. The particle size distribution was determined using an automatic particle identification technique in the Scandium software.

2.1.4. Dynamic light scattering (DLS) and zeta potential.

The particle size of the produced CuONPs was analyzed using a particle size analyzer with a NICOMP 380 ZLS. DLS device (PSS, Santa Barbara, CA, USA), using the 632 nm line of a HeNe laser as the incident light with an angel 90o, while zeta potential was used with an external angel 18.9o at the Nanomaterial Investigation Lab, Central Laboratory Network (CLN), National Research Centre (NRC), Egypt. Three replicated measurements were taken from every sample for greater accuracy.

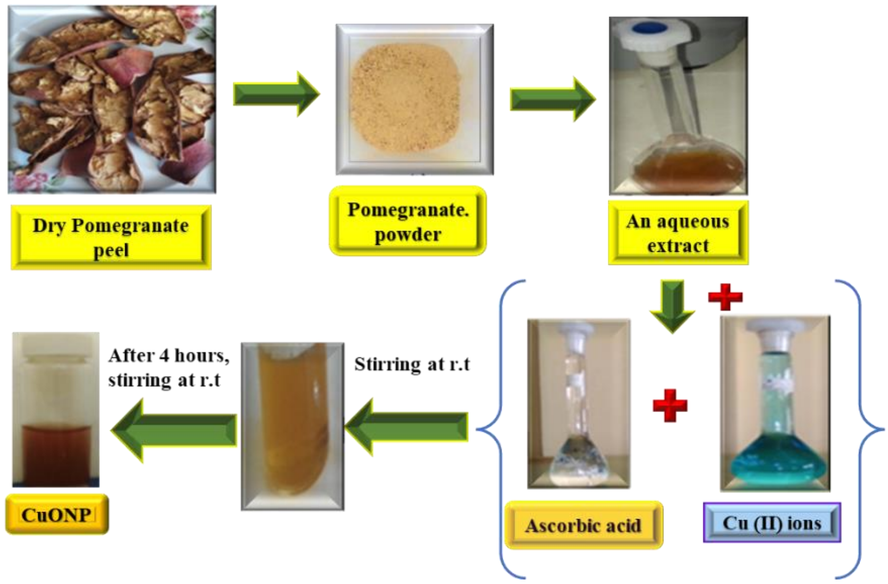
2.2. Plant extraction.

The pomegranate peels were chosen in this study because they were found to be a potential source of bioactive compounds that can be used as a capping agent in CuONPs preparation. Pomegranate (the red type) was collected and obtained from the Sebha farms in southern Libya in February 2023. Fresh pomegranate peels were washed, then chopped into smaller pieces, and dried in a shaded area at room temperature for two weeks. The dried pomegranate peels were ground into a uniform coarse powder using an electric grinder. The plant material was extracted using the maceration method [14, 15] with a slight modification.

A 200 ml aqueous ethanol mixture in a proportion of 6:4 was added to 30 g of grounded dried pomegranate peels in Erlenmeyer flasks (250 ml), and the ratio of plant material mass to solvent volume was 1:4. The mixtures were stirred magnetically, and boiled at 50°C for one hour. The color of the solution changed from watery to yellowish brown. The combined solvent extract was filtered using Whatman filter paper and centrifuged at 1250 rpm for one hour. The pomegranate peel extract was concentrated under reduced pressure (40–50°C) by a rotary evaporator in order to remove any residual EtOH that may still be present in the precipitate. The precipitate is then re-dissolved in distilled water to be used as a capping agent for CuONPs preparation.

2.3. Green synthesis of copper oxide nanoparticles (CuONPs).

CuONP was produced in this study by dissolving 0.05 g of copper chloride ($CuCl_2$) in 25 ml of deionized water and stirring magnetically at room temperature for 5 min. Afterward, pomegranate peel aqueous extract (6 ml) was added to the last mixture while stirring. Once the peel extract comes into contact, copper ions spontaneously change from blue to brownish green. The obtained mixture was left under stirring at room temperature for 2 hours, then the solution of ascorbic acid as a reducing agent 4 ml (0.0018 g in 10 ml H_2O) was added dropwise under strong stirring. The mixture started changing from brownish green to brown suspended, representing the formation of water-soluble monodispersed CuONPs. The reaction was left under stirring for 4 hours in order to ensure the reaction was completed (see Scheme 1).



Scheme 1. The preparation procedure of colloidally dispersed CuONP using an extract of pomegranate peel (as a capping agent) added to the copper chloride solution in the presence of ascorbic acid.

2.4. Antibacterial test.

The synthesized CuONPs from leaf extract were used to evaluate antibacterial tests. Gram-positive *Staphylococcus aureus* (*S. aureus* ATCC 6538P) and Gram-negative *Escherichia coli* (*E. coli* NCIB 8277) were used as bacterial strains. The lowest concentration of a positive antimicrobial agent, which could prevent bacterial growth after overnight incubation, is known as the Minimum Inhibitory Concentration (MIC) assay. The MIC was determined according to the standard protocol used by the Clinical and Laboratory Standards Institute [16]. The McFarland standard was applied to standardize the turbidity of bacterial inoculums. Using a UV-Vis spectrophotometer with a wavelength of 600 nm, the optical density of the bacterial culture cultured in Mueller Hilton broth was adjusted to turbidity corresponding to barium sulfate 0.5 McFarland standards using the Biotek Synergy H1 Hybrid Microplate Reader spectrophotometer at 625 nm. 15 µl of diluted cell suspension was inoculated to 100 µl of Mueller-Hilton broth with a desired concentration (control as without CuONPs, and 8.9 µg /ml, 5.9 µg /ml, 3.9 µg/ml, 2.6 µg/ml CuONPs) in each 96-well plate. Nutrient broth dehydrated powder was used to make the nutrient broth (NB) medium and used for control wells. The culture plate was incubated for 24 h at 37°C. All the experiments were carried out in triplicate, and all the data from the experiments were also analyzed by applying the ANOVA test ($p < 0.05$).

3. Results and Discussion

3.1. Stability of CuONPs.

Several techniques are used to characterize and determine the properties of synthesized CuONPs, which are discussed below.

It is well known that various factors, such as concentration, method of synthesis, pH, temperature, and type of extract, affect the SPR and then influence the properties of synthetic CuONPs [17, 18].

According to Akintelu *et al*., CuONPs have surface plasmon resonance in the range of 200–350nm [8]. This confirms the result in this study, where the surface plasmon resonance (SPR) absorption band was observed at 281 nm for CuONPs using pomegranate peel extract with ascorbic acid at room temperature. UV-visible spectroscopy reveals vital data about the size, stability, agglomeration, and aggregation of the CuONPs [8]. Similar results were illustrated when a dark blue-grey suspension of CuONPs was synthesized using Ganoderma sessile extract, and the absorbance peak was observed at 290.73 nm [19].

The stability of the CuONPs was monitored using the UV-visible technique, where the samples were measured weekly to monitor their stability (see Figure 1). The color changes were the first indicator to produce CuONPs; they were changed from brownish green to brown, confirming the reduction of copper chloride into CuONPs (Cu^{+2} to Cu^0), as mentioned in the earlier study [14].

As can be seen in Figure 1, the size of CuONPs gradually increases when the time is increased. For instance, the SPR of CuONPs produced a single peak centered at 281 nm, which changed to 306 nm after nearly 28 weeks, and there is no sign of any aggregation or agglomeration.

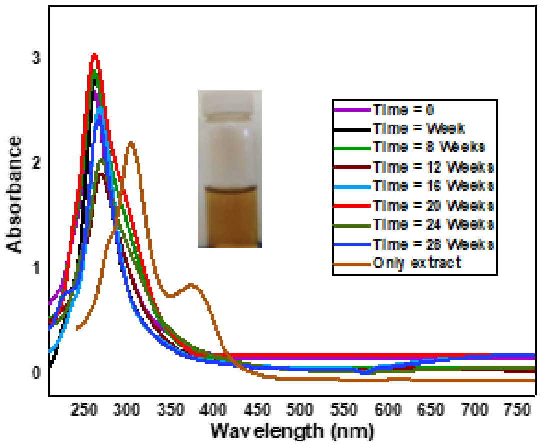


Figure 1. Stability study of CuONPs synthesized by pomegranate peel extract in the presence of ascorbic acid under various time durations: at the initial time (freshly prepared, time =0) and after (from one week to 28 weeks), respectively.

The ascorbic acid was utilized as a reducing agent to aid the extract in regulating the size of CuONPs and enhancing their long-term stability. The SPR of the CuONPs remarkably changed after 28 weeks because the stability of the CuONPs decreased; they became conglomerates, and larger particles formed. In addition, the incubation duration of nanoparticle preparation from plant extract has been investigated to determine how it affects the morphological characteristics and qualities of NPs [8]. The response time of CuONPs is also influenced by additional variables such as storage conditions and light exposure. Particle aggregation and agglomeration have been linked to long incubation times [8]. In this study, the

CuONPs were found to remain stable for more than 6 months due to the presence of repulsive electrostatic forces between the particles and also due to adsorbed ascorbic acid and plant extract on their surface, as referred to in the last investigation [20].

3.2. Effects of experimental parameters on synthesized CuONPs.

In this paper, we study the influence of two key factors, pH and temperature, on the produced CuONPs.

3.2.1. Stability of produced CuONPs under different pH values.

Microenvironment-responsive nanomaterials are attractive for medical applications with regional specificity. It is reported that pH-responsive gold nanoparticles are able to aggregate in an acidic environment that is similar to the cancer environment [21].

The pH-responsive behavior of the particles is derived from the change in electrostatic interaction between the particles, where attraction and repulsion play a significant role in low and high-pH environments, respectively. The difference in particle electrostatic interaction behavior across a range of pH is caused by simple protonation variations and not by irreversible chemical change [21].

A solution's acidity and basicity level can be determined using the pH value. pH changes have been shown to have an impact on the production of CuONPs and other metallic oxide nanoparticles in several investigations [8]. In addition, the important influence of pH on the size, shape, and surface of some nanoparticles biosynthesized from plant extracts has been reported [8]. Although there have been extensive studies on the synthesis of NPs in a variety of pH ranges, particularly on how extremely low and high pH would change the properties of NPs. However, there is little focus on the impact of pH on the aggregation of produced NPs in environmentally relevant conditions. When NPs are exposed to the environment, they may undergo different changes in their state, surface charge, and morphology [22].

The effect of pH on produced NPs has not been vividly investigated until now. For this reason, the stability of CuONPs was investigated at various pH values (0.9 to 12.4) in the basic condition and the acid condition by adding 0.1 M NaOH and 0.1 M HCl (5-50µL)) respectively. The samples were thoroughly mixed for roughly 2 minutes at room temperature (25°C), and the experimental sample was prepared in triplicate for more accuracy.

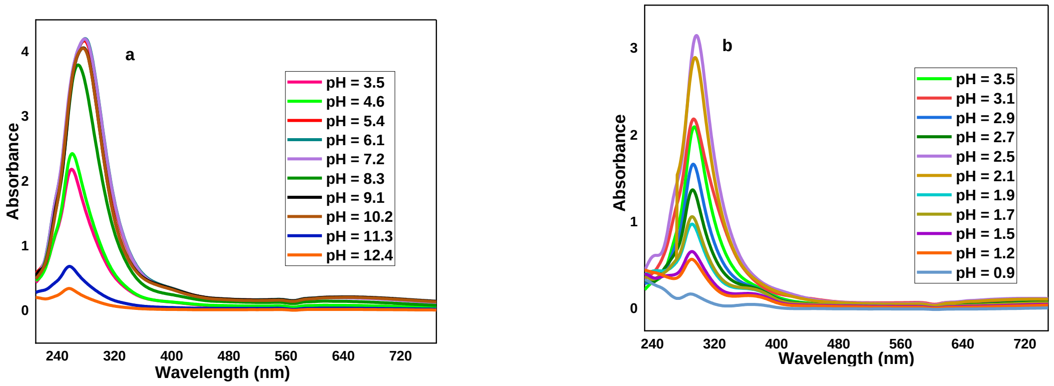


Figure 2. shows the record of UV-Vis measurements at different pH values. Where the pH ranged from 3.5 to 12.4 in basic conditions **(a)** and 0.1 M NaOH was added (5-50 µl). While in acidic conditions **(b)** 0.1 M HCl (5-55 µl) was added. According to the UV-Vis experiment, the measuring plasmonic absorption peaks were completely different when the pH values were changed.

The stability of dispersed CuONPs with changing pH values has decreased at high and low pH values, indicating that large NPs are produced [21]. The red shift in the plasmonic absorption peak (longer wavelength) caused by the low pH-specific aggregation of nanoparticles revealed greater SPR in high acidic pH (1.5-0.9) and high basic pH (8.3-12.4) than in the case of pH 3.5. The SPR of the colloidal CuONPs has been increased from 281 nm to greater than 296 nm with a reducing pH of 3.5 to 0.9. In the same way, when the pH is increased from 3.5 to 12.4, the SPR is also changed from 281 nm to 307 nm, as seen clearly in Figure 2 (a, b).

It is well known that when the pH was lowered to 5.5, which is equivalent to the pH of cancer cells, the particles accumulated into larger plasmonic clusters, as illustrated in the last study [21], which motivates us to verify how CuONPs respond to changes in pH values.

The change in SPR of NPs was noted when the pH values were changed because pH influences the interaction between the functional groups in plant extract and metal ions, as illustrated in earlier investigations [23, 24]. From these findings, the best result and excellent SPR for the synthesis of CuONPs using pomegranate peel extract was obtained at pH = 3.5, and this may be due to the conversion of a large amount of CuCl2 to CuONPs.

3.2.2. Effect of temperature.

One of the most important factors that affect the formation of metallic oxide nanoparticles is temperature. Green synthesis using plant extracts of CuONPs and other metallic oxide nanoparticles should be synthesized at temperatures in the range of 25°C-100°C [8].

It has been revealed that the morphological identity of nanoparticles is affected by increasing temperature [8]. According to research on the green synthesis of metallic oxides made from plant extracts, synthesis occurs quickly and completely at higher temperatures. However, it has been noted that higher temperatures can result in poor nanoparticle formation because they can inactivate the biomolecules responsible for the reduction procedure [8].

In this study, highly stable CuONPs are prepared at room temperature (25°C), which is in agreement with other studies, which illustrated that the green synthesis of CuONPs occurs more frequently at room temperature due to the instability of several secondary metabolites contained in plant extracts [8]. The CuONPs at room temperature showed a sharp and narrow peak, as shown in Figure 3.

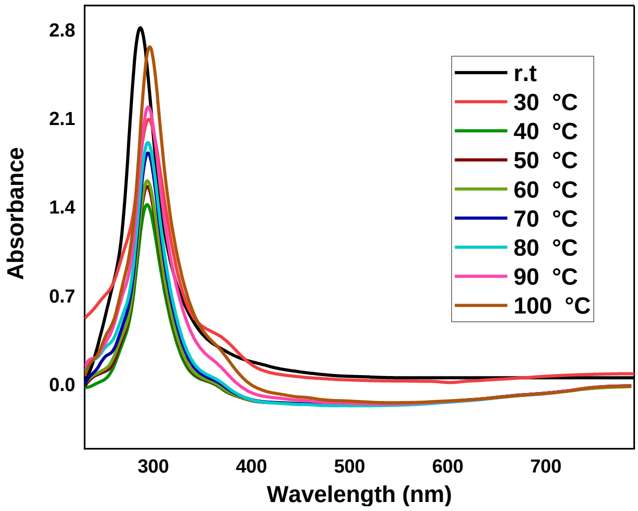


Figure 3. UV–visible absorption spectra of biosynthesized CuONPs using Pomegranate peel extract as a reducing and capping agent. Effects of temperature (30°C to 100°C) on the synthesis of CuONPs.

While, with increasing temperature from 30°C to 100°C of suspending CuONPs, the intensity of the SPR peak is also increased, and a change is observed in the absorption peak position as shown in Table 1. The highest absorbance was seen at 100 °C with a maximum wavelength of 300 nm (see Tale 1), and the absorption intensity was higher than that of other CuONPs at lower temperatures. This clearly reveals that the increasing temperature considerably affects the size of CuONPs [15] and may lead to the agglomeration of CuONPs.

Table 1. The comparison of CuONPs between SPR and their intensity at the range of the temperature from 25°C to 100°C.

Temperature	SPR	Intensity
25°C	281 nm	2.8
30°C	288 nm	2.0
40°C	291 nm	1.4
50°C	292 nm	1.5
60°C	293 nm	1.6
70°C	293 nm	1.7
80°C	294 nm	1.9
90°C	295 nm	2.1
100°C	300 nm	2.5

3.3. Fourier transform infrared spectroscopy (FT-IR) analysis.

FT-IR is used to classify different functional groups associated with the CuONPs surface. In addition, the FT-IR spectrum of pomegranate peel extract displays different absorption peaks, reflecting its complex nature as a result of biomolecules. A broad peak at 3400 cm^{-1} is attributable to the presence of amine NH groups and the O-H group of the overlapping stretching vibration ascribed to H_2O and phenolic compounds present in the extract, which agrees with other studies [14, 25].

A strong absorption peak at wavelength 2900-3000 cm^{-1} was credited to C–H [8]. Figure 4 displays a slight change in both position and intensity in the peaks for the FTIR band of the produced CuONPs. The main characteristic peaks of pomegranate peel extract were observed in the FT-IR spectra of CuONPs, as shown in Figure 4 (a, b).

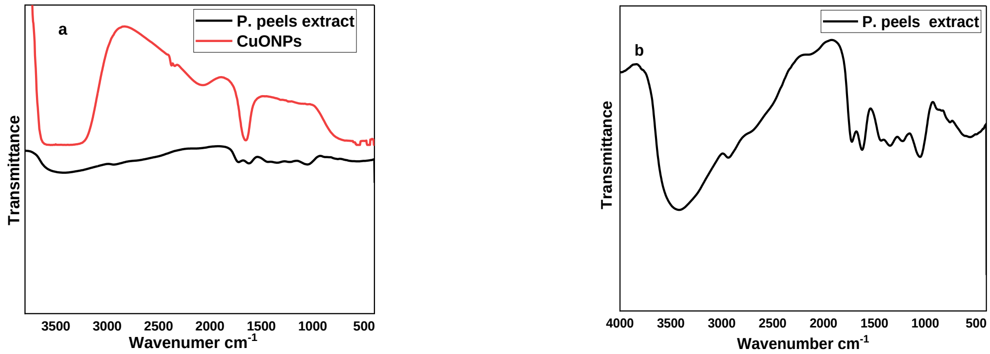


Figure 4. FTIR for the comparison between the pomegranate peel extract as a stabilized ligand and CuONPs prepared from **(a)** the same extract; **(b)** only pomegranate peel extract.

The absorption band observed at wavelength 1737 cm^{-1} is revealed to be C=O [26] of carbonyl, which is absent in the case of CuONPs, and this suggests that pomegranate peel extract can bind to CuONPs via hydroxyl groups and carbonyl of the amino acids on the extracts, as illustrated in an earlier study [6].

Also, the absorption band at 1643 cm^{-1} is due to the C–O group of aliphatic esters in the extract, and this is moved to 1653 cm^{-1} in CuONPs, as shown in Figure 4 (a). Furthermore, the production of CuONP was confirmed via a weak peak at ~550 cm^{-1}, which is due to CuO vibration [27]. Bands at 1010 cm^{-1} and 939 cm^{-1} of CuONPs are attributed to symmetric and asymmetric stretching vibrations of C-O-O bonds [28]. The peaks at 2379 cm^{-1} demonstrate the C≡of alkynes bands, which agrees with the literature [29]. Similar findings have been observed and reported previously for synthesizing CuONPs using J. gendarussa extract [30].

The produced CuONPs showed a new chemical linkage in their FT-IR spectra. This suggests that Pomegranate peel extract can act as a good reducing and stabilizing agent for synthesized CuONPs and prevent their agglomeration, as reported [6].

3.4. Transmission electron microscopy (TEM) results.

TEM is a vital tool for determining the morphology, size, and structural orientation of materials [29, 31]. The characteristic images of the prepared CuONPs are presented in Figure 5 (a, b). The results indicate that the dispersed CuONPs exhibited a spherical shape with different sizes, 19±1.2nm and 54±5 nm at rt and 100°C, respectively.

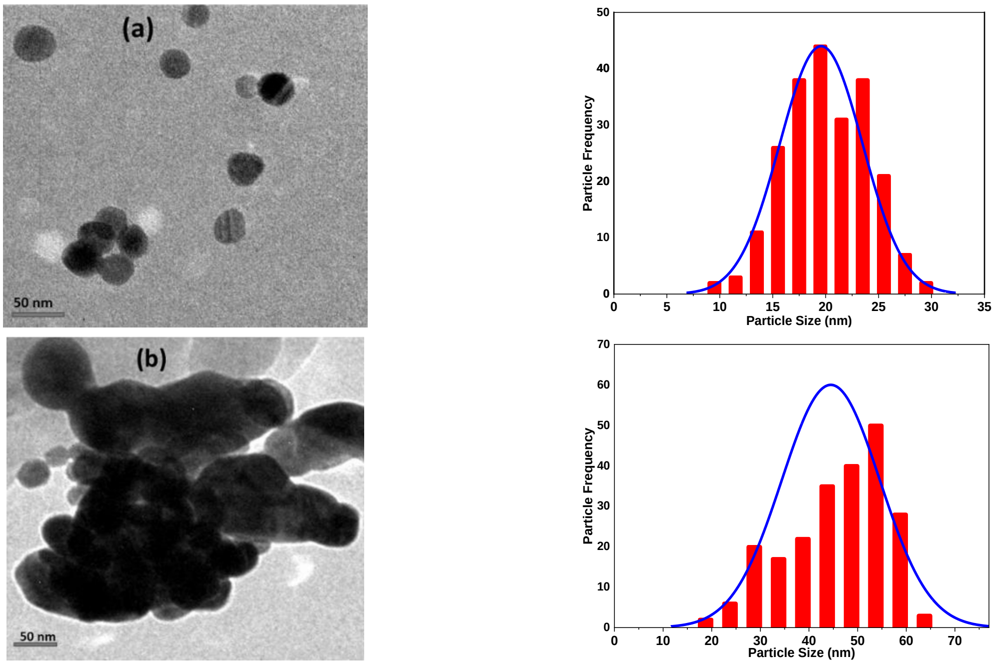


Figure 5. TEM and histogram results of CuONPs at**(a)** room temperature; **(b)** at 100°C.

The increase in size and temperature can be seen, attributed to surface agglomeration, which is visible in Figure 5 (b). While CuONPs displayed uniform size with high stability at room temperature, suggesting strong interactions between the NPs' surface and pomegranate peel extract, as shown in Figure 5(a), this confirms their potential ability to stabilize CuONPs due to their high affinity towards CuONPs. These results are consistent with previously published literature [9, 32].3.5. Dynamic light scattering (DLS) results.

Dynamic light scattering is used to detect the size, size distribution, and hydrodynamic residue of metal and metal oxide nanoparticles [33]. Interestingly, this technique is particularly useful in studying the behavior of NPs in suspensions [33].

In this study, based on the number distribution, DLS found an average size of 33.2±5 nm; this was modified to 98.9±20 nm, which is polydispersible in nature when temperature is increased to 100°C (see Figure 6).

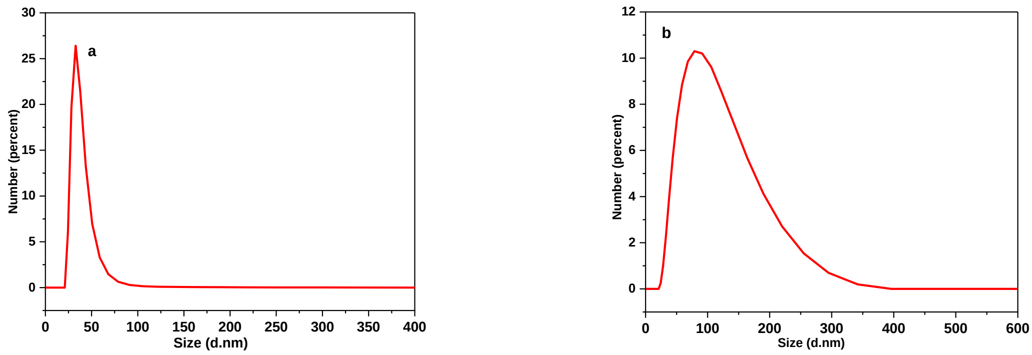


Figure 6. DLS results of CuONPs at **(a)** room temperature; **(b**) at 100°C, respectively.

Regarding the sizes of the CuONPs, the measured size in the case of the DLS technique was quite larger than the size that was obtained from TEM because DLS is used to determine the hydrodynamic diameter of NPs, which are suspended in contact with other available atoms, such as molecules and proteins present in the pomegranate peel extract [34].

3.6. Zeta potential results.

Zeta potential gives serious information on the dispersion of NPs; it measures charges on the nanoparticles' surface, which indicates the mutual repulsion between the particles. The zeta potential of CuONPs dispersed in water was found to be -16.50 ± 1.4 mV (see Figure 7) at a pH of 3.5. The last study showed that CuONP has a negative charge (-13.7 ± 1.55) and was very stable as a result of electrostatic interaction between NPs [35]. The stabilized ligands present in the extract can increase the stability of metal nanoparticles in the long term by preventing probable aggregation [35].

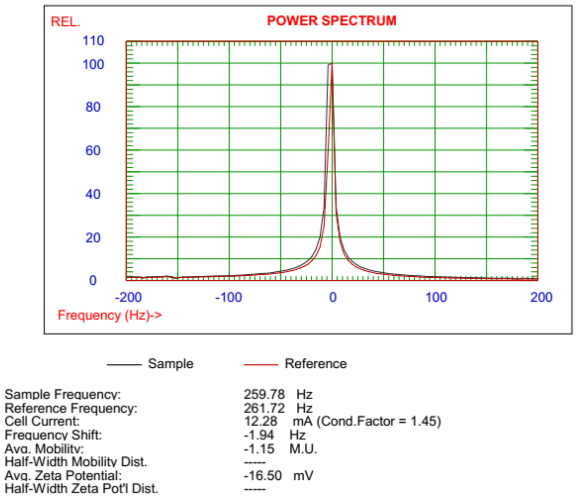


Figure 7. Zeta potential analysis of CuONPs, where the CuONPs were negative in charge with a zeta potential value of −16.50 mV.

The synthesized CuONPs would be stable in storage for a long period due to their greater zeta potential charge [28]. As previously indicated, zeta values show that, based on electrokinetic potential, the stability of NPs is extremely unstable in the range of ±0–10 mV. Conversely, NPs as relatively stable, moderately stable, and extremely stable had zeta values of approximately ±10-20 mV, ±20-30 mV, and more than ±30 mV, respectively [34]. In addition, the average zeta potential of CuONPs ranges from −20 to +45 mV depending on the pH between pH 2 and pH 12, as mentioned in the other study [26]. According to Alhalili, the higher negative zeta potential of CuONPs synthesized by Eucalyptus Globulus leaf extract was

(−16.6 mV) and found to be a strong repulsion force between the particles, which resulted in an enhancement of stability [26], which agrees with our results.

Herein, the zeta potential of biosynthesized CuONPs demonstrates the relative stability of the produced NPs, meaning that the agglomeration will be reduced because the particles will continue to be divergent and in a repulsion state. In addition, using plant extracts as a capping agent for NPs is considered to increase their stability by improving the electrostatic forces between particles [34].

3.7. Antibacterial activities of CuONPs.

Many different leaf extracts, including tea leaf and fruit peel extracts, can be easily used to synthesize CuONPs in a green way. The researchers concentrated on improving the properties of NPs, particularly their capacity to disperse in water and extend their stability in aqueous conditions for use in biological and medical applications [18]. Copper oxide NPs have more antimicrobial effects than zinc oxide and silver oxide NPs against Gram-negative and Gram-positive bacteria [36].

Bacterial and antibiotic resistance are ever-growing global public health problems [28]. The green synthesis of CuONPs has provided a large number of biotechnological and pharmaceutical applications [37]. The synthesis of NPs using plant extract has numerous advantages compared to other biosynthetic processes, such as NPs carried out by bacteria, fungi, actinomycetes, and algae [5]. For example, CuONPs synthesized from plant extracts commonly possess antibacterial properties against various bacteria [19].

The minimum inhibitory concentration (MIC) values of CuONPs against both gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli)* were evaluated in this work to determine the lowest concentration of produced CuONPs that could cause visible growth inhibition of bacteria as shown in Figure 8 (a, b).

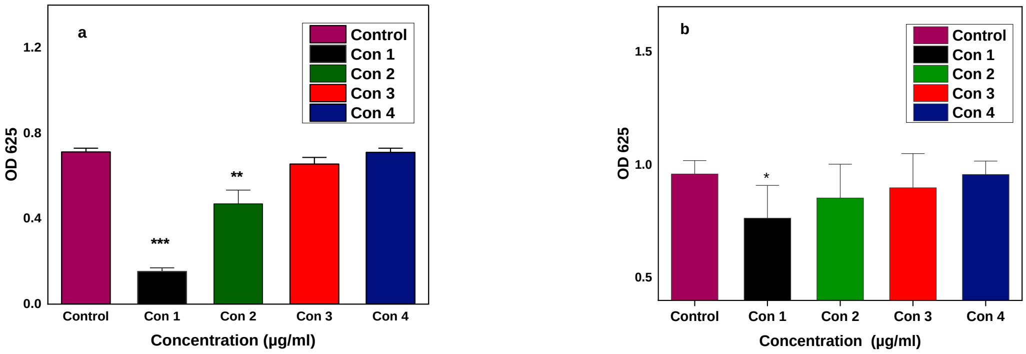


Figure 8. Bar graphs indicate the antibacterial effect of biosynthesized CuONPs using minimum inhibitory concentration (MIC) (control without CuONPs, (8.9 μg/ml, 5.9 μg/ml, 3.9 μg/ml, 2.6 μg/ml) against **(a)** *S. aureus*; **(b)** *E. coli* bacteria. Where (*** represents $p \leq 0.0001$), (** represents $p \leq 0.001$), and (*represents $p \leq 0.05$).

The *E. coli* and *S. aureus* were exposed to various concentrations of CuONPs (8.9 ug/ml, 5.9 ug/ml, 3.9 ug/ml, and 2.6 ug/ml) and were also taken as control-free of CuONPs. The CuONPs have a good inhibitory effect on S. aureus, with a MIC value of 5.9 ug/ml. Where slightly higher concentrations of CuONPs were required. The MIC value of 8.9 ug/ml was observed for *E. coli*.

According to the literature, CuONPs with a negative charge showed more antibacterial activity against Gram-positive bacteria. This increased NPs' ability to enter microbial cells [36], which agrees with our results. This suggests that the CuONPs used in this study are more selective for gram-positive bacterial strains (*S. aureus*) than for gram-negative (*E. coli*), and this study has parallels with others [19].

Pomegranate peel has been widely used in traditional medicine worldwide to treat different types of diseases [38]. Because the peel contains various biologically active compounds, those are evidently responsible for their reportedly higher antibacterial properties [28] than leaves and flowers. For this reason, it was chosen for this investigation because it is a useless portion of the fruit and has the highest antibacterial activity when compared to other pomegranate portions, as reported by Kupnik *et al*. [39].

From this study, it can be confirmed that the pomegranate peel extract used for the green synthesis of CuONPs showed the efficacy of CuONPs at medium and high concentrations, where Staphylococcus aureus exhibited the highest sensitivity to CuONPs. This investigation might encourage scientists to test it as an antibacterial agent against these pathogens.

CuONPs were reported to be more effective than iron oxide nanoparticles and nickel nanoparticles in the fight against multidrug resistance [28].

Herein, we synthesized the CuONPs using an easily simple, safe, and eco-friendly method by using the extract of pomegranate peel as a reducing and stabilizing agent and described their antibacterial activity against S. aureus and E. coli bacteria.

4. Conclusions

The biosynthesis of CuONPs as an environmentally friendly alternative to conventional chemical and physical processes has been explored in this study. Pomegranate peel extract appears to be an alternate source that might be used to make extracellularly stable CuONPs in a straightforward, one-pot, environmentally friendly.

The synthesized CuONPs were characterized by UV-visible, FT-IR, TEM, DLS, and zeta potential. The maximum absorbance was found to be 281 nm for CuONPs using pomegranate peel extract as a reducing and capping agent in the presence of ascorbic acid. The small size with a spherical shape was clearly shown and was 19± 1.2nm based on TEM results. The FTIR spectra confirmed the presence of phenols, carboxylic acids, and amine compounds in the CuONPs sample. The findings are promising and reveal that CuONPs displayed good enough stability with a non-polluting and low-cost method to be used for further bioapplications in the future. These NPs showed antimicrobial activity against S. aureus and E. coli. It could be concluded that pomegranate peel extract is a good candidate for the biosynthesis of CuONPs with tailorable particle size, good morphology, and antibacterial activity.

Funding

The author (s) received no specific funding for this work.

Acknowledgments

The authors express their sincere thanks to London University / UK for some chemical analysis.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Khan, I; Saeed, K; and Khan, I. Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry* **2019**, *12*, 908-931, https://doi.org/10.1016/j.arabjc.2017.05.011.
2. Hajipour, M.J; Fromm, K.M; Ashkarran, A.A; de Aberasturi, D.J; de Larramendi, I.R; Rojo, T; Serpooshan, V; Parak, W.J. and Mahmoudi, M. *Antibacterial properties of nanoparticles.Trends in biotechnology* **2012**, *30*, 499-511, https://doi.org/10.1016/j.tibtech.2012.06.004.
3. Mariadoss, A.V.A.; Saravanakumar, K.; Sathiyaseelan, A.; Venkatachalam, K.; and Wang, M.H. Folic acid functionalized starch encapsulated green synthesized copper oxide nanoparticles for targeted drug delivery in breast cancer therapy. *International Journal of Biological Macromolecules* **2020**, *164*, 2073-2084, https://doi.org/10.1016/j.ijbiomac.2020.08.036.
4. Naz, S; Gul, A; Zia, M; and Javed, R. Synthesis, biomedical applications, and toxicity of CuO nanoparticles. *Applied Microbiology and Biotechnology* **2023**, *107*, 1039-1061. https://doi.org/10.1007/s00253-023-12364-z.
5. Liu, Y.; Zeng, Z.; Jiang, O.; Li, Y.; Xu, Q.; Jiang, L.; Yu, J. and Xu, D. Green synthesis of CuO NPs, characterization and their toxicity potential against HepG2 cells. *Materials Research Express* **2021**, *8*, 015011, https://doi.org/10.1088/2053-1591/abd666.
6. Vasantharaj, S.; Sathiyavimal, S.; Saravanan, M.; Senthilkumar, P.; Gnanasekaran, K.; Shanmugavel, M.; Manikandan, E.; and Pugazhendhi, A. Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: characterization of antibacterial activity and dye degradation potential. *Journal of Photochemistry and Photobiology B: Biology* **2019**, *191*, 143-149, https://doi.org/10.1016/j.jphotobiol.2018.12.026.
7. Khan, F.H. Chemical hazards of nanoparticles to human and environment (a review). *Oriental Journal of Chemistry* **2013**, *29*, 1399. http://dx.doi.org/10.13005/ojc/290415.
8. Akintelu, S.A.; Folorunso, A.S.; Folorunso, F.A.; and Oyebamiji, A.K. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. *Heliyon* **2020**, *6*, e04508, https://doi.org/10.1016/j.heliyon.2020.e04508.
9. Antonio-Pérez, A.; Durán-Armenta, L.F.; Pérez-Loredo, M.G. and Torres-Huerta, A.L. Biosynthesis of Copper Nanoparticles with Medicinal Plants Extracts: From Extraction Methods to Applications. *Micromachines* **2023**, *14*, 1882, https://doi.org/10.3390/mi14101882.
10. Barathikannan, K.; Venkatadri, B.; Khusro, A.; Al-Dhabi, N.A.; Agastian, P.; Arasu, M.V.; Choi, H.S. and Kim, Y.O. Chemical analysis of Punica granatum fruit peel and its in vitro and in vivo biological properties. *BMC complementary and alternative medicine* **2016**, *16*, 1-10, https://doi.org/10.1186/s12906-016-1237-3.
11. Andishmand, H.; Azadmard-Damirchi, S.; Hamishekar, H.; Torbati, M.; Kharazmi, M.S.; Savage, G.P.; Tan, C. and Jafari, S.M. Nano-delivery systems for encapsulation of phenolic compounds from pomegranate peel. *Advances in Colloid and Interface Science* **2023**, *311*, 102833, https://doi.org/10.1016/j.cis.2022.102833.
12. Ain, H.B.U.; Tufail, T.; Bashir, S.; Ijaz, N.; Hussain, M.; Ikram, A.; Farooq, M.A. and Saewan, S.A. Nutritional importance and industrial uses of pomegranate peel: A critical review. *Food Science & Nutrition* **2023**, *11*, 2589-2598, https://doi.org/10.1002/fsn3.3320.
13. Bertolo, M.R.; Martins, V.C.; Plepis, A.M.G. and Junior, S.B. Utilization of pomegranate peel waste: Natural deep eutectic solvents as a green strategy to recover valuable phenolic compounds. *Journal of Cleaner Production* **2021**, *327*, 129471, https://doi.org/10.1016/j.jclepro.2021.129471.
14. Ghidan, A.Y.; Al-Antary, T.M. and Awwad, A.M. Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: Effect on green peach Aphid. *Environmental Nanotechnology, Monitoring & Management* **2016**, 6, 95-98, http://dx.doi.org/10.1016/j.enmm.2016.08.002.
15. Rajeshkumar, S.; Lakshmi, T.; Tharani, M. and Sivaperumal, P. Green synthesis of gold nanoparticles using pomegranate peel extract and its antioxidant and anticancer activity against liver cancer cell line. *Alinteri Journal of Agricultural Sciences* **2020**, *35*, 164-169.
16. Asimuddin, M.; Shaik, M.R.; Adil, S.F.; Siddiqui, M.R.H.; Alwarthan, A.; Jamil, K. and Khan, M. Azadirachta indica based biosynthesis of silver nanoparticles and evaluation of their antibacterial and cytotoxic effects. *Journal of King Saud University-Science* **2020**, *32*, 648-656, https://doi.org/10.1016/j.jksus.2018.09.014.
17. Mohamed, E.A. Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. *Heliyon* **2020**, *6*, e03123, https://doi.org/10.1016/j.heliyon.2019.e03123.

18. Siddiqui, V.U.; Ansari, A.; Chauhan, R. and Siddiqi, W.A. Green synthesis of copper oxide (CuO) nanoparticles by Punica granatum peel extract. Materials Today: *Proceedings* **2021**, *36*, 751-755, https://doi.org/10.1016/j.matpr.2020.05.504.
19. Flores-Rábago, K.M.; Rivera-Mendoza, D.; Vilchis-Nestor, A.R.; Juarez-Moreno, K. and Castro-Longoria, E. Antibacterial Activity of Biosynthesized Copper Oxide Nanoparticles (CuONPs) Using Ganoderma sessile. *Antibiotics* **2023**, *12*, 1251, https://doi.org/10.3390/antibiotics12081251.
20. Pal, B; Rana, S. and Kaur, R. Influence of different reducing agents on the Ag nanostructures and their electrokinetic and catalytic properties. *Journal of Nanoscience and Nanotechnology* **2015**, *15*, 2753-2760, https://doi.org/10.1166/jnn.2015.9222.
21. Park, S.; Lee, W.J.; Park, S.; Choi, D.; Kim, S. and Park, N. Reversibly pH-responsive gold nanoparticles and their applications for photothermal cancer therapy. *Scientific reports* **2019**, *9*, 20180, https://doi.org/10.1038/s41598-019-56754-8.
22. Fernando, I. and Zhou, Y. Impact of pH on the stability, dissolution and aggregation kinetics of silver nanoparticles. *Chemosphere* **2019**, *216*, 297-305, https://doi.org/10.1016/j.chemosphere.2018.10.122.
23. Akintelu, S.A.; Oyebamiji, A.K.; Olugbeko, S.C. and Latona, D.F. Green chemistry approach towards the synthesis of copper nanoparticles and its potential applications as therapeutic agents and environmental control. *Current Research in Green and Sustainable Chemistry* **2021**, *4*, 100176, https://doi.org/10.1016/j.crgsc.2021.100176.
24. Kurian, J.T; Chandran, P. and Sebastian, J.K. Synthesis of inorganic nanoparticles using traditionally used Indian medicinal plants. *Journal of Cluster Science* **2023**, *34*, 2229-2255, https://doi.org/10.1007/s10876-022-02403-6.
25. Dhir, S.; Dutt, R.; Singh, R.P.; Chauhan, M.; Virmani, T.; Kumar, G.; Alhalmi, A.; Aleissa, M.S.; Rudayni, H.A. and Al-Zahrani, M. Amomum Subulatum Fruit Extract Mediated Green Synthesis of Silver and Copper Oxide Nanoparticles: Synthesis, Characterization, Antibacterial and Anticancer Activities. *Processes* **2023**, *11*, 2698, https://doi.org/10.3390/pr11092698.
26. Alhalili, Z. Green synthesis of copper oxide nanoparticles CuO NPs from Eucalyptus Globoulus leaf extract: Adsorption and design of experiments. *Arabian Journal of Chemistry* **2022**, *15*, 103739, https://doi.org/10.1016/j.arabjc.2022.103739.
27. Tshireletso, P.; Ateba, C.N. and Fayemi, O.E. Spectroscopic and antibacterial properties of CuONPs from orange, lemon and tangerine peel extracts: *Potential for combating bacterial resistance. Molecules* **2021**, *26*, 586, https://doi.org/10.3390/molecules26030586.
28. Ibrahem, N.; Latif, H.; M Saifeif, M. and Mogazy, A. Impact of Different Crystal Sizes of Nano-Iron Oxide as Fertilizer on Wheat Plants Photosynthetic Pigments Content. *Egyptian Journal of Chemistry* **2021**, *64*, 4635-4639, https://doi.org/10.21608/ejchem.2021.67176.3447.
29. Abhimanyu, P.; Arvind, M. and Kishor, N. Biosynthesis of CuO Nanoparticles Using Plant Extract as a Precursor: Characterization, Antibacterial, and Antioxidant Activity. *Nano Biomedicine and Engineering* **2023**, *15*, 369-377, https://doi.org/10.26599/NBE.2023.9290027.
30. Vasantharaj, S; Shivakumar, P; Sathiyavimal, S; Senthilkumar, P; Vijayaram, S; Shanmugavel, M. and Pugazhendhi, A. Antibacterial activity and photocatalytic dye degradation of copper oxide nanoparticles (CuONPs) using Justicia gendarussa. *Applied Nanoscience* **2023**, *13*, 2295-2302, https://doi.org/10.1007/s13204-021-01939-9.
31. Xing, J.; Xian, H.; Yang, Y.; Chen, Q.; Xi, J.; Li, S.; He, H. and Zhu, J. Nanoscale Mineralogical Characterization of Terrestrial and Extraterrestrial Samples by Transmission Electron Microscopy: A Review. *ACS Earth and Space Chemistry* **2023**, *7*, 289-302, https://doi.org/10.1016/j.lithos.2023.107113.
32. Balaji, T.; Manushankar, C.M.; Al-Ghanim, K.A.; Kamaraj, C.; Thirumurugan, D.; Thanigaivel, S.; Nicoletti, M.; Sachivkina, N. and Govindarajan, M. Padina boergesenii-Mediated Copper Oxide Nanoparticles Synthesis, with Their Antibacterial and Anticancer Potential. *Biomedicines* **2023**, *11*, 2285, https://doi.org/10.3390/biomedicines11082285.
33. Jia, Z.; Li, J.; Gao, L.; Yang, D. and Kanaev, A. Dynamic Light Scattering: A Powerful Tool for In Situ Nanoparticle Sizing. *Colloids and Interfaces* **2023**, *7*, 15, https://doi.org/10.3390/colloids7010015.
34. Eid, A.M.; Fouda, A.; Hassan, S.E.D.; Hamza, M.F.; Alharbi, N.K.; Elkelish, A.; Alharthi, A. and Salem, W.M. Plant-Based Copper Oxide Nanoparticles; Biosynthesis, Characterization, Antibacterial Activity, Tanning Wastewater Treatment, and Heavy Metals Sorption. *Catalysts* **2023**, *13*, 348, https://doi.org/10.3390/catal13020348.

35. Çalhan, S.D. and Gündoğan, M. Copper oxide nanoparticles: synthesis, characterization, antimicrobial activities and catalytic reduction of methylene blue. *Journal of the Turkish Chemical Society Section A: Chemistry* **2020**, *7*, 561-570, https://doi.org/10.18596/jotcsa.650993.
36. Mohammadi, A.H.; Sobhani-Nasab, A.; Nejati, M.; Hadi, S.; Behjati, M.; Mirzaii-Dizgah, I.; Hasan-Abad, A.M. and Karami, M. Preparation and characterization of CuO, Ag2O and ZnO nanoparticles and investigation of their antibacterial and anticancer properties on HCT-116 and C26 cells. *Inorganic Chemistry Communications* **2023**,*149*, 110404, https://doi.org/10.1016/j.inoche.2023.110404.
37. Thandapani, G.; Arthi, K.; Pazhanisamy, P.; John, J.J.; Vinothini, C.; Rekha, V.; Santhanalakshmi, K. and Sekar, V. Green synthesis of copper oxide nanoparticles using Spinacia oleracea leaf extract and evaluation of biological applications: Antioxidant, antibacterial, larvicidal and biosafety assay. *Materials Today Communications* **2023**, *34*, 105248, https://doi.org/10.1016/j.mtcomm.2022.105248.
38. Abu-Dalo, M.; Jaradat, A.; Albiss, B.A. and Al-Rawashdeh, N.A. Green synthesis of TiO2 NPs/pristine pomegranate peel extract nanocomposite and its antimicrobial activity for water disinfection. *Journal of Environmental Chemical Engineering* **2019**, *7*, 103370, https://doi.org/10.1016/j.jece.2019.103370.
39. Kupnik, K.; Primožič, M.; Vasić, K.; Knez, Ž. and Leitgeb, M. A Comprehensive Study of the Antibacterial Activity of Bioactive Juice and Extracts from Pomegranate (Punica granatum L.) Peels and Seeds. *Plants* **2021**, *10*, 1554, https://doi.org/10.3390/plants10081554.